mine deoxyribose from the standard curve (diphenylamine method), total phosphate (Allen method), labile phosphate (H_3PO_4 liberated on action of adenylpyrophosphatase prepared from potato was determined), and H_3PO_4; adenine : deoxyribose : total phosphate : labile phosphate : inorg. phosphate = 1 : .087 : 3.2 : 2.14 : 0 (Calcd. 1 : 1 : 3 : 2 : 0). Paper electrophoresis of the dATP fraction exhibited a single ultraviolet-absorbing spot.

The authors are grateful to Dr. S. Kuwada, Director of the Laboratories, and Dr. S. Tatsuoka, Vice-Director of the Laboratories, for permission for the publication of the experimental results. The authors are also indebted to members of the Technical Department of this Company for carrying out enzymic assays and pyrophosphate estimation.

Summary

Reaction of the dicyclohexylguanidinium salt of adenosine 5′-phosphoramidate (I) with tribenzyl pyrophosphate (II) in *o*-chlorophenol, and reductive debenzylation of the reaction mixture resulted in the formation of adenosine 5′-triphosphate (ATP). The guanidinium salt was reacted with bis-triethylammonium pyrophosphate (V) in a mixture of tricresol and acetonitrile, and ATP was isolated as its barium salt from the reaction mixture by ion-exchange chromatography. The overall yield of ATP from (I) was 43%.

Reaction of the dicyclohexylguanidinium phosphoramidates of uridine, cytidine, and desoxyadenosine with (V) also gave uridine, cytidine, and desoxyadenosine 5′-triphosphates in a good yield.

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37. Mikio Honjo, Yoshiyasu Furukawa, Kin-ichi Imai, Hiroki Moriyama, and Kuniyoshi Tanaka : Synthesis of Uridine Diphosphate-Glucuronic Acid.*1

(*Research Laboratories, Takeda Chemical Industries, Ltd.**2)

Since UDPGA*3 was discovered[1~4] in the liver as a factor promoting the formation of conjugated glucuronic acid, it has been found by many workers that this substance is a biochemically important compound, which participates not only in detoxication as the active form of glucuronic acid but also in the synthesis of various polysaccharides. Up to now, UDPGA has been isolated in very small quantities from the liver of rabbits[4] and guinea pigs,[5] or from mung bean seedlings,[6] or prepared enzymically from UDPG and

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*1 Brief communication published in this Bulletin, 8, 750 (1960).

*2 Juso-Nishino-cho, Higashiyodogawa-ku, Osaka (本庄美喜男, 古川純康, 今井欣一, 森山博規, 田中邦喜).

*3 Abbreviations used : UDPGA, uridine diphosphate glucuronic acid; UDPG, uridine diphosphate glucose; DPN, diphosphopyridine nucleotide; UTP, uridine 5′-triphosphate; GA-1-P, glucuronic acid 1-phosphate; UMP-NH_2, uridine 5′-phosphoramidate; UMP, uridine 5′-phosphate; DUPP, P^1,P^2-diuridine 5′-pyrophosphate; UDP, uridine 5′-diphosphate; DCC, dicyclohexylcarbodiimide.

1) G. J. Dutton, I. D. E. Storey : Biochem. J., **48**, xxix (1951).
2) *Idem* : *Ibid.*, **53**, xxxvii (1953).
3) *Idem* : *Ibid.*, **57**, 275 (1954).
4) *Idem* : *Ibid.*, **57**, 279 (1954).
5) E. E. B. Smith, G. T. Mills : Biochim. et Biophys. Acta, **13**, 386 (1954).
6) J. Solms, W. Z. Hassid : J. Biol. Chem., **228**, 357 (1957).

DPN in the presence of dehydrogenase[7] or from UTP and GA-1-P by the action of pyrophosphorylase,[8] but no method has so far been established for the chemical synthesis of this compound.*4 In addition, as the 1-position of glucuronic acid may take α- or β-configuration and as the configuration at this position in natural UDPGA has not yet been established, the synthesis of both isomers was attempted in order to clarify the configuration of natural UDPGA.

First, the synthesis of α-UDPGA was attempted by condensation of UMP-NH_2 (I) with α-GA-1-P (II). As a preliminary experiment, the dicyclohexylguanidinium salt[9] of (I) was allowed to react with the triethylammonium salt[10,11] of (II) at 20° in pyridine or *o*-chlorophenol, and part of the reaction mixture was investigated by paper electrophoresis (pH 3.9). As a result, it was found that a new faint ultraviolet-absorbing spot presumed to be due to UDPGA (III) appeared at a site nearer the anode than the UDPG used as control, and that the spot became more intense gradually with time and most after 7~9 days. On the other hand, it was also observed that as UMP-NH_2 was decomposed to UMP, the spot of the latter became more intense gradually, and the reaction temperature of 37° was the most favorable for the formation of (III) than 20° or 30°. Consequently, a solution of the dicyclohexylguanidinium salt of (I) and the triethylammonium salt of (II) in pyridine was left standing at 37° for 10 days and, after distilling off the solvent from the reaction mixture, an aqueous solution of the residue was subjected to chromatography on Dowex-1, X 8 (Cl-form). The resin was first treated with 0.003*N* hydrochloric acid + 0.01*M* sodium chloride to elute UMP and UMP-NH_2 and then with 0.003*N* hydrochloric acid + 0.06*M* sodium chloride, whereupon a substance assumed to be UDPGA was eluted but it was contaminated with the simultaneously produced DUPP. Therefore, the resin, after treating with 0.003*N* hydrochloric acid + 0.01*M* sodium chloride, was eluted with 0.003*N* hydrochloric acid + 0.04*M* sodium chloride to give a mixture of DUPP and an unidentified substance, and then with 0.003*N* hydrochloric acid + 0.1*M* sodium chloride, when a substance presumed to be UDPGA was washed out (Fig. 1). The last fraction was adsorbed on active charcoal and eluted with

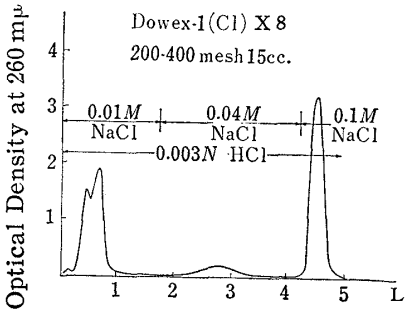

Fig. 1.

hydr. ethanol containing ammonia and the eluate was concentrated in a reduced pressure. That this is a solution of pure UDPGA is evident from the following tests: (a) paper electrophoresis (pH 3.9) of the solution gave a single ultraviolet-absorbing spot at $R_{UMP\text{-}NH_2}$*5 = 1.9; (b) paper partition chromatography of the solution showed the same Rf value as that of authentic UDPGA (UDPG was oxidized with dehydrogenase in the presence of DPN

*4 While this full paper was prepared for publication, synthesis of UDPGA was reported by S. Roseman, J. J. Distler, J. G. Moffatt, and H. G. Khorana (J. Am. Chem. Soc., 83, 659 (1961)).
*5 Ratio of the migration distance of the sample divided by that of UMP-NH_2.

7) J. L. Strominger, E. S. Maxwell, J. Axelroad, H. M. Kalcker: *Ibid.*, **224**, 79 (1957).
8) D. S. Feingold, E. F. Neufeld, W. Z. Hassid: Arch. Biochem. Biophys., **78**, 401 (1958).
9) R. W. Chambers, J. G. Moffatt: J. Am. Chem. Soc., **80**, 3752 (1958).
10) C. A. Marsh: J. Chem. Soc., **1952**, 1578.
11) S. A. Barker, E. J. Bourne, J. G. Fleetwood, M. Stacey: *Ibid.*, **1958**, 4128.

Table I. Analysis of α- and β-UDPGA Fractions

	Ratio (referred to uridine)		
	Found		Calcd. for UDPGA
	α	β	
Uridine	1	1	1
Total Phosphate[a)]	1.992	2.08	2
Acid-labile phosphate[b)]	0.945	1.02	1
Inorganic phosphate[a)]	0	0	0
Glucuronic acid[c)]	0.893	1.04	1
Reducing value[d)]	0	0.1	0
Reducing value after hydrolysis[b)]	0.885	1.01	1

a) R. J. L. Allen: Biochem. J., **34**, 858 (1940).
b) Hydrolysis with 1*N* HCl for 10 min. at 100°.
c) Z. Dische: J. Biol. Chem., **167**, 189 (1947).
d) J. J. Park, W. J. Johnson: *Ibid.*, **181**, 149 (1949).

and the reaction mixture*6 was treated to separate the pure UDPGA); (c) the analytical values of the α-UDPGA fraction were as shown in Table I; (d) paper electrophoresis and paper partition chromatography of the hydrolysate obtained by heating an aliquot of the solution with 0.1*N* hydrochloric acid at 100° for 10 minutes detected the formation of UDP (and UMP), showing the presence of a pyrophosphate bond and at the same time that the carboxyl group of the glucuronic acid is free, and therefore no phosphate combination was effected at that group, and (e) pKa (3.2) of the solution also showed the presence of one free carboxyl group.

The substance eluted with 0.003*N* hydrochloric acid + 0.04*M* sodium chloride was a mixture of DUPP and an unidentified substance, and from the behavior of the latter to paper electrophoresis (pH 5.0), it was assumed at first to be the structural isomer (Ⅷ) of UDPGA, but later examinations showed that the unidentified substance contained no glucuronic acid and behaved completely in the same manner as authentic UDP in paper electrophoresis (pH 7.5), and therefore it must be UDP.

In the condensation between UMP-NH_2 (Ⅰ) and α-GA-1-P (Ⅱ), if the phosphoric acid of (Ⅰ) combines with the carboxyl group of (Ⅱ) as $-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-O-\overset{\overset{O}{\|}}{C}-$ instead of $-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-O-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-$, the formation of (Ⅶ) is expected as a matter of course, but (Ⅶ) was not produced even as a by-product. This fact may be explained as follows: If (Ⅶ) is unstable, it would be decomposed into UMP and (Ⅱ), or if the electronegativity of O^- of phosphoric acid is stronger than that of O^- of a carboxyl group, nucleophilic attack would be favorably effected at P^+ of phosphoramidate by phosphoryl O^- and the pyrophosphate bond is formed.

Addition of a barium acetate solution and ethanol to the above concentrated α-UDPGA fraction precipitated the barium salt, which was purified into a colorless crystalline powder. Paper electrophoresis of the product showed a single spot corresponding to UDPGA (*Anal.* Calcd. for $C_{15}H_{19}O_{18}N_2Ba_{1.5}$: C, 22.90; H, 2.42; N, 3.56; P, 7.89. Found: C, 22.73; H, 2.71; N, 3.39; P, 7.61).

Next, the dicyclohexylguanidinium salt of (Ⅰ) was condensed with the triethylammonium salt of β-GA-1-P[12] (Ⅳ) in the same manner as in the case of α-compound to give a solution of pure β-UDPGA (Ⅴ). The solution behaved completely like the α-

*6 This sample was given by Dr. Hatanaka of the Biochemical Department, School of Medicine, Kyoto University.

12) O. Touster, V. H. Reynolds: J. Biol. Chem., **197**, 863 (1952).

compound in paper electrophoresis and paper partition chromatography, and its analytical values are shown in Table I. Paper electrophoresis and paper partition chromatography of the hydrolysate obtained by heating the solution with 0.1*N* hydrochloric acid at 100° for 10 minutes indicated simultaneous formation of UDP and UMP. Judging from the above results, the solution must be of β-UDPGA.

Synthesis of α-UDPGA was also attempted by another method. Oxygen was introduced into an aqueous solution of the sodium salt of α-UDPG (VI) at 40° for 10 hours in the presence of a platinum catalyst and paper electrophoresis of the reaction mixture distinctly showed the presence of α-UDPGA (III) besides UDPG. On the other hand, UMP and the triethylammonium salt of α- or β-GA-1-P were reacted with DCC in aqueous pyridine, and paper electrophoresis of the reaction mixture gave a faint ultraviolet-absorbing spot corresponding to UDPGA, but this method was given up because the simultaneous formation of DUPP was great and the yield of UDPGA was less than that in the amidate method.

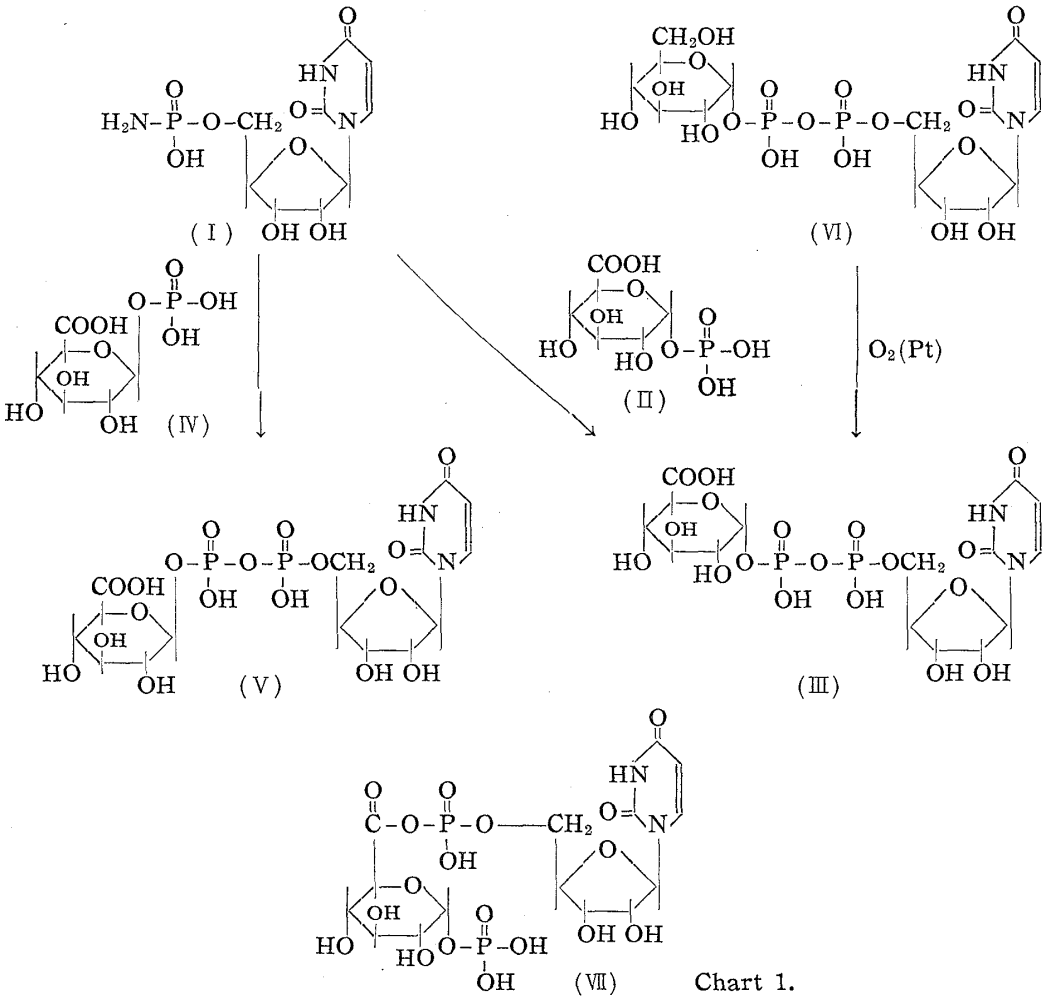

Chart 1.

The α- and β-UDPGA solutions thus obtained, as well as the above mentioned authentic UDPGA solution, were assayed for their ability to form *o*-aminophenol glucuronide, using the glucuronyltransferase in microsomes of the liver of a guinea pig[7] and it was found that α-UDPGA prepared by the two methods was active, but the β-compound was inactive, while the authentic UDPGA was naturally active. This fact indicated that natural UDPGA takes α-configuration and that oxidation of α-UDPG to UDPGA by UDPG-dehydrogenase is not accompanied by Walden inversion, but the inversion is effected in the formation of β-glucuronide from natural UDPGA by the transferase.

Experimental

Triethylammonium Salt of α-GA-1-P (II)——A solution of 0.5 g. of the tripotassium-salt of (II) in 5 cc. of H_2O was passed through a column of 5 cc. of Amberlite IR-120 (H-form) and the column was washed with H_2O until the effluent became neutral. To the solution of free (II) thus obtained (20 cc.; content of GA-1-P, 1.01 m*M* from analytical value of P), 0.45 cc. of Et_3N was added, and the mixture was evaporated *in vacuo* to leave a syrupy substance, which was dried over P_2O_5 and used in the next experiment.

Triethylammonium Salt of β-GA-1-P (IV)——To a solution of 320 mg. of the barium salt of (IV) (purity, 57.4%) in 5 cc. of H_2O a mixture of 1.0 cc. of 2*N* H_2SO_4 and 0.35 cc. of Et_3N was added, the resulting $BaSO_4$ was centrifuged and washed with H_2O, the supernatant was combined with the washing (content of GA-1-P, 0.301 m*M* from analytical value of P) and evaporated *in vacuo*, leaving a syrupy substance.

Reaction between the 1,3-Dicyclohexylguanidinium Salt of UMP-NH_2 (I) and the Triethylammonium Salt of α-GA-1-P (II)——A solution of 206 mg. (0.22 m*M*) of the dicyclohexylguanidinium salt of (I) (purity, *ca.* 60%), dried at 100° for 2 hr., and the above-mentioned triethylammonium salt of (II), each dissolved in 10 cc. of dehyd. pyridine, were mixed and allowed to stand at 37°. After 2, 5, 8, and 10 days, part of the reaction mixture was examined by paper electrophoresis (pH 3.9; AcOH-AcONa buffer; 11 v./cm.; 2.5 hr.), and it was found that the ultraviolet-absorbing spot at R_{UMP-NH_2} 2 enlarged with time, a faint ultraviolet-absorbing spot also appeared at R_{UMP-NH_2} 1.6, and the reaction was optimal at 37°. Use of *o*-chlorophenol instead of pyridine gave the same result.

Separation by Ion-exchange Resin——Pyridine was distilled off from the above reaction mixture, a solution of the residue in 38 cc. of H_2O was poured on a column of 15 cc. of Dowex-1, X8 (Cl-form; 200~400 mesh), and the column, after washing with H_2O, was eluted with 0.003*N* HCl containing NaCl. The chromatography was carried out at 3°.

Fraction	1	0.003*N* HCl + 0.01*M* NaCl	800 cc.	total optical density 950
〃	2	〃 + 0.04*M* 〃	2200 〃	〃 300
〃	3	〃 + 0.10*M* 〃	630 〃	〃 1160

Fraction 3 was poured on a column of 6 g. of active charcoal, the column was washed with a small amount of H_2O, eluted with 95 cc. of 50% EtOH containing 0.5% of NH_4OH, (total optical density 850), and the eluate was concentrated to 4.5 cc. in a reduced pressure. Fractions 1 and 2 were treated in the same manner.

Paper Electrophoresis of Each Concentrated Fraction——Paper electrophoresis (0.1*M* acetate buffer; pH 3.9; 11 v./cm.; 2.5 hr.) of Fraction 3 exhibited an ultraviolet-absorbing spot at R_{UMP-NH_2} 1.9 and that of Fraction 1 showed the spots of the unchanged UMP-NH_2 and UMP. Paper electrophoresis (0.1*M* phosphate buffer; pH 7.5; 11 v./cm.; 3 hr.) of fraction 2 gave an ultraviolet-absorbing spots at R_{UMP} 0.8 and 1.3.

Hydrolysis of Concentrated Fraction 3 with Hydrochloric Acid——Fraction 3 was hydrolyzed by heating with 0.1*N* HCl at 100° for 10 min. Paper electrophoresis (0.1*M* acetate buffer; pH 5.0; 11 v./cm.; 2.5 hr.) of the hydrolysate gave two ultraviolet-absorbing spots at R_{UMP} 1.0 and 1.6, and the latter spot was in accord with that of authentic UDP. Paper partition chromatography [1*M* $AcONH_4$ (pH 7.5)-95% EtOH=3:7.5; 20°; 18 hr.; Whatman No. 1; descending method] of the same hydrolysate indicated two ultraviolet-absorbing spots at $R_{adenosine}$ 0.63 and 0.53, which were in accord with those of UMP and UDP, respectively.

pKa of Concentrated Fraction 3——Taking the molecular extinction coefficient of UDPGA for 9.9×10^3, its 0.01*M* solution was prepared on the basis of the absorption at 260 mμ, and 10 cc. of the

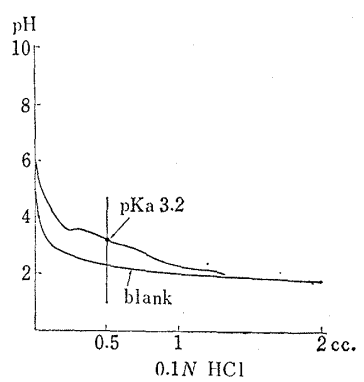

Fig. 2.

solution was subjected to potentiometric titration with 0.1*N* HCl (pH meter with glass electrodes, manufactured by Leeds & Northrup Co.), finding the presence of one acid group having pKa 3.2. Reverse titration of the titrated solution with 0.1*N* NaOH also gave the same value (Fig. 2).

Identification of Concentrated Fraction 2——A sample was applied on filter paper in a line and submitted to paper electrophoresis (0.1*M* phosphate buffer; pH 7.5; 11 v./cm.; 3 hr.), giving two ultraviolet-absorbing bands at R_{UMP} 0.8 and 1.3. Each of the bands was cut out and extracted by standing with 0.1*N* HCl overnight. Naphthoresorcinol and carbazole reactions of the extracts showed absence of glucuronic acid, but the above-mentioned absorbing bands were at the same sites as those of authentic DUPP and UDP, respectively.

Isolation of α-UDPGA-Ba Salt——To 4.5 cc. of the concentrated α-UDPGA fraction obtained as above a solution of barium acetate [$(AcO)_2Ba \cdot H_2O$, 70 mg.+H_2O, 0.5 cc.] was added and the mixture was decolorized with 50 mg. of charcoal. EtOH was added gradually to the solution until 50% in volume, the resulting white precipitate was dissolved in 6 cc. of H_2O, and the solution was centrifuged to remove a small amount of an insoluble substance. To the supernatant 4 cc. of EtOH was added and the resulting precipitate, after washing with 10 cc. of 40% EtOH, was dried over P_2O_5 to give 37.7 mg. of a white crystalline powder. Paper electrophoresis (0.1*M* acetate buffer; pH 3.9; 11 v./cm.; 2.5 hr.) of the product gave an ultraviolet-absorbing spot. *Anal.* Calcd. for $C_{15}H_{19}N_2O_{18}P_2Ba_{1.5}$: C, 22.90; H, 2.42; N, 3.56; P, 7.89. Found: C, 22.73; H, 2.71; N, 3.39; P, 7.61.

Reaction between the 1,3-Dicyclohexylguanidinium Salt of UMP-NH_2 (I) and the Triethylammonium Salt of β-GA-1-P (IV)——Solutions of 67.2 mg. (0.07 m*M*) of the dicyclohexylguanidinium salt of (I), (purity, ca. 60%), dried at 100° for 2 hr., and the triethylammonium salt of (IV) mentioned above, each dissolved in 5 cc. of dehyd. pyridine, were mixed, left standing at 37°, and the reaction mixture was examined by paper electrophoresis after 5, 7, and 9 days. There was no difference in the ultraviolet-absorbing spots ($R_{UMP\text{-}NH_2}$ 2) after 7 and 9 days.

Separation with Ion-exchange Resin——The above reaction mixture was evaporated to dryness *in vacuo*, the residue was dissolved in 13 cc. of H_2O, the solution was adjusted to pH 8.5 with dil. NH_4OH, and adsorbed on a column of 5 cc. of Dowex-1, X8 (Cl-form; 200~400 mesh). The column was treated in the same manner as in the case of α-compound. About 400 cc. (total optical density 370) of fraction 3 (0.003*N* HCl+0.10*M* NaCl) thus obtained was poured on a column of 1.6 g. of charcoal, the column was washed with H_2O, and eluted with 33 cc. of 50% EtOH containing 0.5% of NH_4OH (total optical density 307). The eluate was concentrated and examined by paper electrophoresis, detecting one ultraviolet-absorbing spot at the same site as α-compound. The analytical values of the solution are given in Table I.

Oxidation of α-UDPG (VI)——O_2 was passed through a solution of 20 mg. of the sodium salt of (VI) (synthetic) in 5 cc. of H_2O for 6 hr. at room temperature in the presence of Pt catalyst, prepared from 13 mg. of PtO_2, but the starting material alone was recovered from the reaction mixture (decomposition of UDPG was not observed by paper electrophoresis). The catalyst was filtered off and O_2 was passed through the filtrate, after addition of 1 cc. of H_2O, at 40° for 8.5 hr. in the presence of a catalyst prepared from 30 mg. of PtO_2. Paper electrophoresis of the reaction mixture indicated the formation of UDPGA.

Isolation of α-UDPGA from the Enzymic Oxidation Product of α-UDPG (VI)——About 10 μ*M* of (VI) was oxidized by UDPG-dehydrogenase in the presence of DPN. The reaction mixture (1 cc.) was diluted with 40 cc. of H_2O, adsorbed on a column of 1 g. of charcoal, and the column was eluted with 60 cc. of 50% EtOH containing 0.5% of NH_4OH (total optical density 420). The eluate was concentrated, applied on Toyo filter paper No. 5B in a line, and subjected to paper electrophoresis (0.1 *M* $AcONH_4$ buffer; pH 5.2; 11 v./cm.; 3 hr.), showing four ultraviolet-absorbing spots. The foremost spot, which was in accord with synthetic α-UDPGA in migration distance, was cut out and extracted with 30 cc. of H_2O at 0° overnight. The extract (total optical density 29.4) was adsorbed on 50 mg. of charcoal, eluted with 5 cc. of 50% EtOH containing 0.5% of NH_4OH, and the eluate was concentrated to 1.2 cc. *in vacuo* (total optical density 23.5) to furnish a solution of standard α-UDPGA for paper partition chromatography and enzymic assay.

Paper Partition Chromatography of α-UDPGA——The above-mentioned standard α-UDPGA was compared with concentrated fraction 3 by three kinds of paper partition chromatography and they showed the same migration. Filter paper; Whatman No. 1 (prewashed with 2*N* AcOH). (a) $AcONH_4$ (pH 7.5)-95% EtOH (7.5:3); descending method; 16 hr. R_{AMP} 1, (b) Iso-PrOH-1% $(NH_4)_2SO_4$ (6:4); ascending method; 24 hr.; Rf 0.31, (c) MeOH-H_2O-conc. NH_4OH (6:3:1); ascending method; 16 hr.; R_{AMP} 1.2.

Enzymic Assay of UDPGA

Assay Method——To a mixture of 0.2 cc. of 0.5*M* tris buffer (pH 7.5), 0.1 cc. of 0.3*M* $MgCl_2$ solution, 0.1 cc. of 0.015*M* ammonium saccharate solution, 0.1 cc. of the test solution (adjusted to contain 0.1 μ*M* UDPGA), and 0.1 cc. of *o*-aminophenol+ascorbic acid (a solution of 6.8 mg. of pure *o*-aminophenol and 50 mg. of ascorbic acid in 25 cc. of H_2O) 0.3 cc. of H_2O was added and the whole was preincu-

bated at 37° for 5 min. To the preincubated solution, 0.1 cc. of the enzyme solution (the suspension of 105,000 *g* fraction in 10 cc. of isotonic KCl solution, prepared from 10 g. of the liver of a guinea pig) was added and further incubated at 37° for 15 min. with occasional shaking. One cc. of a mixture of 1.25*M* TCA and 1*M* H_3PO_4 (pH 2) was added to the resulting solution, the mixture was centrifuged, and 1.0 cc. of the supernatant was diazotized with 0.2 cc. of 0.05% $NaNO_2$ solution. The excess HNO_2 was decomposed with 0.2 cc. of 0.5% ammonium sulfamate solution, 0.2 cc. of 0.1% naphthylenediamine hydrochloride solution was added to the reaction mixture, and the whole was allowed to stand at 37° for 2 hr. If *o*-aminophenolglucuronide is formed, the reaction mixture turns pink.

The authors are grateful to Dr. S. Kuwada, Director of the Laboratories, and Dr. S. Tatsuoka, Vice-Director of the Laboratories, for their generosity in permitting the publication of this paper. Thanks are also due to Prof. O. Hayaishi and Dr. K. Hatanaka of the Biochemical Department, School of Medicine, Kyoto University, for their kindness in giving a sample of UDPGA prepared enzymically and the aid of enzymic assay. The authors are also indebted to Dr. Y. Asahi for the measurement of pKa and to Mr. M. Kan and his associates for microanalyses.

Summary

Uridine 5′-phosphoramidate (I) was allowed to react with α-glucuronic acid 1-phosphate (II), and α-uridine diphosphate glucuronic acid (α-UDPGA) (III) was isolated from the reaction mixture by ion exchange chromatography. Likewise, β-UDPGA (V) was produced from (I) and β-glucuronic acid 1-phosphate (IV). Formation of (III) was also observed by the oxidation of α-uridine diphosphate glucose (VI) in the presence of platinum oxide catalyst. Ability of (III) and (V) to form *o*-aminophenolglucuronide was examined with the transferase in microsomes of a guinea pig liver ane only (III) was found to be active. This fact indicated that natural UDPGA takes the same configuration as (III).

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38. Yasushi Sanno and Kuniyoshi Tanaka : A New Method for Synthesis of Cytidine Diphosphate-Ethanolamine and Cytidine Diphosphate-Choline.*1

(*Research Laboratories, Takeda Chemical Industries, Ltd.**2)

Cytidine diphosphate (CDP)-choline (IX) and cytidine diphosphate (CDP)-ethanolamine (IV) are coenzymes playing an important rôle in the metabolism of phospholipids.

Both compounds were discovered and the mechanism of their metabolism was clarified in 1956 by Kennedy, *et al.*[1] In the same year, Kennedy, *et al.* synthesized[2] (IX) and (IV) by condensation of cytidine 5′-monophosphate (5′-CMP) (VII) with choline phosphate (X) or with ethanolamine phosphate[3] (VI) in hydrous pyridine in the presence of dicyclohexylcarbodiimide (DCC) (V). This DCC method was first duplicated[2] (Chart 1) and it was found that although CDP-choline (IX) could be obtained in a relatively good yield as

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*1 Y. Sanno, K. Tanaka : This Bulletin, 8, 753 (1960).
*2 Juso-Nishino-cho, Higashiyodogawa-ku, Osaka (三野 安, 田中邦喜).
1) E. P. Kennedy, S. B. Weiss : J. Biol. Chem., **222**, 193 (1956).
2) E. P. Kennedy : *Ibid.*, **222**, 185 (1956).
3) V. Ferrari, G. Ferrari : Arch. sci. biol. (Bologna), **37**, 1 (1953); (C. A., **47**, 11534e (1953)).